

# PCR Cleanup

Add 5 times the volume of PCR sample of PB (B stands for binding)

To make even more of the gene of interest, we did (and should do) 2 PCR reactions.  
You can combine those products for this.

Identical to miniprep from now on

1. Using micropipet, add remaining PCR product (stuff that was not used in gel) into column
  - a. centrifuge 13,000 RPM for 1 min
  - b. pour off flow-through in liquid waste
2. wash with alcohol PE buffer to keep plasmid stuck on filter
  - . check the cap sticker to make sure ethanol is added
- a. add 750 uL PE buffer to column filter
- b. centrifuge 13,000 RPM for 1 min, no liquid should be left on filter
- c. pour off Ethanol flow-through in collection tube into liquid waste
3. evaporate alcohol by spin
  - . centrifuge 13,000 RPM for 1 min
4. add Elution Buffer (EB: H<sub>2</sub>O+ salts) to filter to elute DNA
  - . move filter over brand new Eppendorf 1.5mL tube
  - . add 50 uL (35 uL if you want it more concentrated) EB to filter
- a. Let SIT ON BENCH on filter for 1 min
- b. centrifuge 13,000 RPM for 1 min